Remarks

Claims 1, 3-10, 12, 13, 22, 23 and 27-29 are pending in this application. Claims 1, 7, 10 and 28 are amended to correct form. New claim 30 is added herein that parallels pending claim 29 but depends from clam 28.

No new matter is added herein. Reconsideration of the application is respectfully requested in view of the foregoing amendments and following remarks.

Rejections under 35 U.S.C § 103 (a)

Claims 1, 3-10, 12, 13, 22, 23, 27 and 28 are rejected under 35 U.S.C. § 103(a) as allegedly being obvious Lee et al. in view of Economides et al. (U.S. Patent No. 6,586,251) and Meyers et al. (Nature Genetics, 18: 136-141, published February 1998). Applicants respectfully disagree with this rejection.

MPEP § 2143 sets for that the differences must be ascertained between the claimed invention and the prior art. In the present case, the prior art differs substantially from the claimed methods, as the use of two selectable markers, each flanked by a pair of recombining sites, is not suggested by the cited prior art. In fact, the constructs disclosed in the cited prior art could not achieve the desired effect if two different recombinases were used with the disclosed construts. Thus, the Lee et al., Economides et al., and Meyers et al., either alone or in any combination, does not render obvious the claimed methods.

Lee et al. teach a de-repressible promoter (pL) operably linked to a nucleic acid encoding Beta, Exo and Gam under the control of a temperature sensitive repressor. Lee et al. teach that this method can be used to introduce the arabinose promoter operably linked to Cre, and describe the introduction of a FRT-Kan-FRT cassette into a gene in a bacterial artificial chromosome. As confirmed Lee et al. do not suggest, or render obvious inserting a second nucleic acid encoding a selectable marker flanked by a pair of second recombining sites into a second site in the gene. In addition, Lee et al. do not suggest, nor render obvious, excising the nucleic acid encoding the selectable marker with a second recombinase specific for the second recombining sites, wherein

two first recombining sites remain in the gene following excision of the nucleic acid encoding the selectable marker, and wherein recombination of the two first recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein.

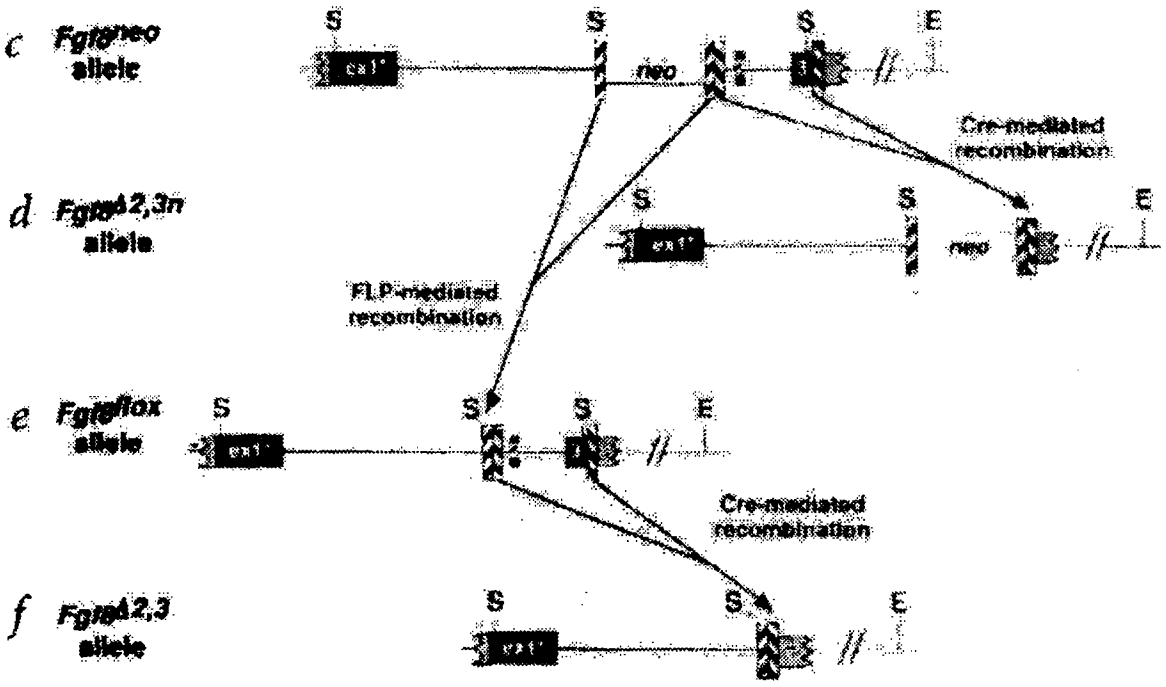
A Declaration of E-Chiang Lee (the first author of Lee et al.) and Pentao Liu under 37 C.F.R. § 1.132 (hereinafter the Declaration) was submitted previously and is of record. This Declaration described the work performed by Drs. Lee and Liu. The Declaration delineates the dramatic differences between Lee et al. and the presently claimed methods, namely the methods described in Lee et al. do not use a second set of recombining sites flanking a second selectable marker, and they do not provide any information on how to produce a nucleic acid sequence that **cannot be transcribed into a functional protein.**

Indeed, the intent of Lee et al. is to induce a nucleic acid encoding functional Cre protein into an untranslated region of a gene (Eno2), such that Cre is expressed in a tissue specific manner (along with the Eno2 protein). *Thus, Lee et al. describes a method for introducing a functional gene in a regulatory element so that the encoded protein is properly expressed. The methods described in Lee et al. are completely opposite from the presently claimed methods of generating vectors for the knockout of a gene, wherein recombination of the recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein, as required in claim 1.*

Economides et al. describe methods for using large DNA vectors to target, via homologous recombination, chromosomal loci in eukaryotic cells. These large DNA targeting vectors are termed LTVECs, and include large fragments of cloned genomic DNA. These LTVECs can be used to delete a coding region (column 3). As described in column 10, the modification of a region in the LTVEC can result in the creation of conditional alleles. Specifically, it is disclosed that a conditional allele can be generated "by introduction of loxP sites flanking the region to be excised by Cre recombinase [citation deleted] or FRT sites flanking the region to be excised by Flp recombinase." Thus, Economides teach that that a single pair of recombining sites can be used to produce a LTVEC that can conditionally "knock-out" a section of an LTVEC (up to many tens of kilobases, see column 10, line 18). Economides do not teach the use of a second pair of recombining sites flanking a selectable marker, nor do they teach any specific steps in a method for producing the construct.

Meyers et al. teach a strategy producing an Fgf8 allele that can be converted to either a null allele or a hypomorphic allele. To produce this mouse line, a targeting vector is constructed that can be used to either delete Fgf8 or produce alternative splicing of Fgf8. It is important to note that these methods do not use bacterial cells, let alone bacterial cells that express Exo or Beta. This vector can be used to create transgenic mice.

In the hypomorphic allele disclosed in Myers et al., two loxP sites flank a coding sequence (exons 2 and 3) essential for the production of functional Fgf8 and two FRT sites flank a neo gene. When mice carrying the hypomorphic allele are mated to mice expressing Cre, recombination at the LoxP sites produces a non-functional Fgf8 gene (since exons 2 and 3 are deleted and the retention of the neo gene results in altered transcription). This is shown in scenario D in the figure below.



Meyers et al. discloses that the neo gene includes a splice donor and acceptor; this results in altered Fgf8 mRNA when a neo gene is inserted into the coding sequence. The inclusion of the neo gene results in the production of alternative transcripts that produce a different Fgf8 protein. When mice carrying the hypomorphic allele are mated to mice that express FLP (scenario e), there is no alternative splicing of Fgf8.

When mice are utilized that are not mated to animals that include FLP, the mice include the neo gene (scenario c). In heterozygous mice, the level of Fgf8 transcirpts is reduced by more than 50%. Specifically, half of the mRNA encoding Fgf8 is abnormal (due to the inclusion of neo such that the splicing is altered), but half of FgF8 transcripts are functional (see page 138, first paragraph). These mice have abnormalities "consistent with an impairment of Fgf8 gene function."

To determine if the effects of knocking out the gene will be more severe with both altered transcription and a knock out, mice carrying the allele are mated such that Cre recombination occurs (scenario d) are mated to mice with a functional Fgf8 allele. These mice were compared to heterozygous mice that have the knockout only (scenario f, knock-out without altered transcription) and a wild type allele. Meyers et al. determined that the combination of altered transcription (the neo gene) and the knock-out results in an augmented effect.

It is important to note that the studies presented in Myers et al. are designed to determine the effect of altered splicing (through the inclusion of a neo cassette) and knock-out of the Fgf8 gene alone and in combination. In order to be able to evaluate both effects using a single construct, only a single neo gene flanked by a pair of recombining sites can be included. If two copies of a neo gene were included within the construct, then altered splicing would occur in the presence of either FLP or Cre. Thus, the construct would be completely ineffective to achieve the desired result. Contrary to the assertions in the Office action on pages 8-9, Myers et al. *teach away* from a construct including a first selectable marker flanked by a pair of first recombining sites and a second selectable marker flanked by a second pair of recombining sites.

As discussed above, Lee et al. and Economides et al. disclose only the inclusion of a single selectable marker flanked by pair of recombining sites. Even if one were to combine Lee et al. and Economides et al. with Meyers et al., one of skill in the art simply would not arrive at the claimed methods. Specifically, one of skill in the art would produce only constructs with a single pair of recombining sites flanking a selectable marker. Lee et al., Economides et al. and Meyers et al., even in combination, simply do not suggest: (1) the use of two selectable markers, each flanked by a pair of recombining sites; or (2) any specific steps in any method that would lead to a vector for a conditional knockout, wherein two selectable markers are utilized.

While it might be argued that the "mere existence of differences between the prior art and an invention does not establish the invention's nonobviousness" *Dann v. Johnston*, 425 U.S. 219,

230, 189 USPQ 257, 261 (1976), it is not just the differences that support the non-obviousness of the claimed methods. Meyers et al. teach that there are the substantial effects caused by the inclusion of a selectable marker in a construct. The constructs for the production of a hypomorphic allele disclosed in Meyers et al. would be completely ineffective if a second selectable marker were included between the second pair recombining sites. Thus, the teachings of Meyers et al., while not only different from the present claimed method, provide evidence of the non-obviousness of producing a construct including a second pair of recombining sites flanking a second selectable marker for the production of a vector for the conditional knock-out of a gene.

Moreover, with regard to claim 28 and claims that depend therefrom, it is unclear how Lee et al., Economides et al., and Meyers et al. teach the claimed methods. Lee et al. and Economides et al. only teach the use of a single set of recombining sites flanking a selectable marker. Meyers et al. teach methods and constructs that require the use of one selectable marker; the insertion of another neo gene between the LoxP sites would negate the usefulness of the constructs described by Meyers et al. In addition, the prior art does not suggest the production of constructs that have a single LoxP site and a single FRT site in a bacterial artificial chromosome, wherein the nucleic acid further comprises a LoxP site 3' of the FRT site. The prior art simply cannot be combined or construed to teach the claimed steps in the method. Thus, there simply is no *prima facie* case of obviousness.

The MPEP further sets forth that objective evidence relevant to the issue of obviousness must be evaluated. Such evidence, sometimes referred to as "secondary considerations," may include evidence of commercial success, long-felt but unsolved needs, difficulties in producing the claimed invention, and unexpected results. As a secondary consideration, the Applicants note that *a large number of experiments were required to devise the presently claimed methods*. The Examiner does not appear to have considered the additional information in the Declaration. Dr. Lee was not able to simply use the work he presented in Lee et al. to quickly devise methods for producing vectors for conditional knockouts. The large number of the required experiments is evidenced in data and methods presented the Declaration of Dr. Liu under 37 C.F.R. § 1.132. Applicants request that the Office consider this evidence as a secondary consideration, namely difficulties in producing the claimed invention, see MPEP § 2145.

Claim 29 is rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Lee et al. in view of Economides et al. and Meyers et al. and further in view of Stewart et al. (U.S. Patent No. 6,586,251).

Lee et al., Economides et al. and Meyers et al are discussed above.

Stewart et al. disclose methods for homologous recombination in bacterial cells, and describe cloning and subcloning and documenting that homologous recombination can be used to introduce a single selectable marker. Applicants agree that Stewart et al. teach the use of Rec E/T and Red α/β to induce homologous recombination, and teach that homologous recombination can be achieved on bacterial artificial chromosomes. Stewart et al. do teach the use of homology arms.

However, there is no description in Stewart et al. of any specific steps in a method for generating conditional targeting vectors. Stewart et al. does not describe: (1) the use of two selectable markers, each flanked by a pair of recombining sites; (2) the use of homologous recombination to make vectors for conditional knock-outs; or (3) specific steps in any method that would lead to a vector for a conditional knockout, let alone a method that includes the use of both LoxP sites and FRT sites, both Cre and FLP, and results in vectors that include a specific configuration of LoxP and FRT sites. Thus, Stewart et al. simply cannot be construed to make up the substantial deficiencies of Lee et al., Economides et al. or Meyers et al. A *prima facie* case of obviousness cannot be established based on the cited prior art.

Reconsideration and withdrawal of the rejection is respectfully requested.

Claim Objections

Claim 28 is objected to for including typographical errors. Claims 6 and 10 are objected to under 37 C.F.R. 1.75(c), as being of improper dependent form. Claims 6, 10 and 28 are amended herein as suggested in the Office action, rendering these objections moot.

Conclusion and Formal Request for Interview

Applicants believe the present application is ready for allowance, which action is requested. If any issues remain prior to the issuance of a Notice of Allowance, or if the Examiner has any questions regarding Declaration of Dr. Liu under 37 C.F.R. § 1.132 or the Declaration under 37 C.F.R. § 1.131, or if the Examiner has any objections to the pending

claims, the Examiner is formally requested to contact the undersigned using the telephone number provided below prior to issuance of the any final Office Action in order to arrange a telephonic interview. It is believed that a brief discussion of the merits of the present application may expedite prosecution. This request is being submitted under MPEP § 713.01, which indicates that an interview may be arranged in advance by a written request.

Respectfully submitted,

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